Published in Antiviral Research, volume 105, May 2014, pages 92–99.

http://dx.doi.org/10.1016/j.antiviral.2014.02.020

Are statins a viable option for the treatment of infections with the hepatitis C virus?

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Keywords: HCV, statins, cholesterol, antiviral, prenylation, liver, combination therapy

Abstract

Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors that are widely used for the treatment of hypercholesterolemia. Besides their cholesterol-lowering effect, statins have been reported to have antiviral activity against a variety of viruses, including hepatitis C virus (HCV). Several statins inhibit the *in vitro* replication of subgenomic HCV replicons and also suppress *in vitro* RNA replication of infectious HCV. The precise mechanism of the anti-HCV activity of statins has not yet been defined. Recent studies suggest that the antiviral effect may result from the inhibition of geranylgeranylation of cellular proteins rather than the inhibition of cholesterol synthesis. Despite the antiviral effect observed *in vitro,* statin monotherapy seems to be insufficient for the treatment of chronic HCV infection. However, several prospective and retrospective studies demonstrated that the addition of statins to IFN-α and ribavirin therapy increases SVR, RVR, and EVR rates without the occurrence of additional adverse events. These clinical data, together with the excellent safety profile and low cost, suggest that statins may play a role in HCV therapy until more potent and safe direct-acting antivirals become available.

Introduction

Over the last decade, major progress has been made in the discovery of novel antivirals for the treatment of hepatitis C virus (HCV) infections. In 2011 the first HCV protease inhibitors, telaprevir and boceprevir, were approved in combination with pegylated interferon (PEG-IFN)-α and ribavirin (RBV) for the treatment of chronic hepatitis C genotype 1 infections in Europe and the United States. A number of other direct-acting antivirals (DAA) are in clinical developmental either in combination with PEG-IFN-α and RBV or with other DAAs in IFN-free regimens (with or without RBV). However, the use of DAAs may be hampered by (severe) side-effects, the development of resistant variants and (for some) lack of efficacy against genotypes other than genotype 1. Furthermore, novel DAA combination therapies will probably not be affordable in the developing world in the foreseeable future. The addition of low-cost drugs such as statins could thus improve the efficacy of traditional PEG-IFN/RBV therapy without high additional costs. Several studies have investigated the potential antiviral activity of statins in patients chronically infected with HCV. To date, the clinical data on the efficacy of statin therapy in HCV-infected patients are conflicting, making it difficult to estimate the potential use of statins in future HCV therapy. In this review, the current state of the art on the *in vitro* and *in vivo* anti-HCV activity of statins is summarized and the potential of statins in the treatment of chronic HCV infection is discussed.

Statins

Statins are the second most commonly prescribed drugs worldwide and are mainly used for primary and secondary prevention of cardiovascular disease. Besides their cholesterol-lowering effect, statins also have anti-proliferative, pro-apoptotic, anti-angiogenic, immunomodulatory and anti-infective effects. Statins inhibit 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme involved in cholesterol biosynthesis in the liver. HMG-CoA reductase catalyzes the conversion of HMG-CoA into mevalonic acid (see Figure 1). Therefore, the inhibition of HMG-CoA reductase by statins results in the reduction of several downstream products of the

mevalonate pathway, such as cholesterol, but also prenyl precursors like farnesyl and geranylgeranyl pyrophosphate. During post-translational modification steps these prenyl precursors are covalently attached to various cellular proteins to facilitate their membrane association.

Statins have been reported to possess *in vitro* antiviral activity against a variety of viruses, such as HIV-1 [(Giguère and Tremblay, 2004)(del Real et al., 2004)(Amet et al., 2008)], poliovirus (Liu et al., 2006), cytomegalovirus (Potena et al., 2004), dengue virus (Martínez-Gutierrez et al., 2011) and respiratory syncytial virus (Gower and Graham, 2001). Furthermore, a recent review highlights the possibility of administration of statins to reduce mortality in the case of an influenza pandemic (Fedson, 2013). Several statins were also shown to inhibit the replication of subgenomic HCV replicons (Ikeda et al., 2006) and to suppress RNA replication of infectious HCV (Amemiya et al., 2008). The observed anti-HCV activity of statins suggests that HCV requires elements of the cholesterol biosynthetic pathway for efficient replication. In other studies it was demonstrated that HCV has an intimate link with the host lipid metabolism (recently reviewed in (Schaefer et al, 2013)). HCV virions circulate in the blood in complex with lipoproteins (Thomssen et al., 1992) and lipoprotein receptors such as the LDL receptor and SR-BI have been reported to be involved in HCV entry. Furthermore, HCV exploits the VLDL assembly and secretion pathway to be released from hepatocytes and lipid droplets (LDs) play an important role in HCV virion assembly.

The mechanism by which statins inhibit HCV replication is still unknown. The anti-HCV activity of statins does not seem to be related to their cholesterol-lowering ability since fluvastatin appears to be the most active statin in inhibiting *in vitro* HCV replication (Sezaki et al., 2009) but inhibits HMG CoA reductase to a lesser extent than other statins (IC_{50} of 28 nM vs 5-11 nM for atorvastatin, simvastatin, cerivastatin and rosuvastatin) (Istvan and Deisenhofer, 2001). Furthermore, adding exogenous cholesterol does not reverse the *in vitro* antiviral effect of statins, whereas addition of exogenous geranylgeranyl pyrophosphate (GGpp) does (Kapadia et al, 2005). This confirms that the anti-HCV activity of statins is not due to the inhibition of cholesterol synthesis but due to the inhibition of geranylgeranylation of cellular proteins. F-box and leucine rich repeat protein 2 (FBL2)

was identified as a geranylgeranylated host factor essential for HCV replication (Wang et al., 2005). FBL2 belongs to the family of the F-box proteins and is likely involved in an ubiquitination reaction, but its substrate(s) are unknown (16). At this moment it is not entirely clear if and how FBL2 is required for HCV replication and whether it is fully responsible for the antiviral effect of the statins.

***In vitro* anti-HCV activity of statins**

The anti-HCV activity of statins was first described in 2003 (Ye et al., 2003). HCV genotype 1 replicon containing cells were treated with lovastatin to study the involvement of protein and sterol membrane modifications in viral RNA replication and formation of the viral replication complex. Administration of 50 µM of lovastatin resulted in a reduction in HCV RNA levels of 70-95%.

In 2006 the different anti-HCV profiles of five statins were characterized (atorvastatin, fluvastatin, lovastatin, pravastatin and simvastatin) (Ikeda et al., 2006). To this end, the OR6 full length replicon system (genotype 1b) was used (Ikeda et al., 2005). A range of anti-HCV activities was described with fluvastatin being the most active inhibitor in the series (IC_{50} = 0.9 µM). Lovastatin, for which anti-HCV activity had been demonstrated earlier [(Ye et al., 2003), (Kapadia and Chisari, 2005)], had a more modest antiviral effect (IC_{50} = 2.2 µM). In contrast, pravastatin, which is also a known inhibitor of HMG-CoA reductase, did not inhibit HCV replication, suggesting a more complex mechanism of action than the one previously suggested, namely the inhibition of cholesterol biosynthesis. Importantly, the authors also demonstrated that combination treatment of fluvastatin and IFN-α resulted in a synergistic inhibitory effect on the levels of HCV RNA replication in the HCV genotype 1 replicon system. These data suggest that statins could be used as part of a combination therapy with the current standard of care. The *in vitro* activity of a similar panel of statins (comprising fluvastatin, atorvastatin, mevastatin, simvastatin, lovastatin and pravastatin) was characterized in 2007 (Kim et al., 2007). We further confirmed the anti-HCV activity of several statins (lovastatin, mevastatin, simvastatin and fluvastatin) in genotype 1b subgenomic replicon cells as well as the JFH1-CS/N6 HCVcc system (Delang et al., 2009).

Based on the aforementioned studies and depending on the selected *in vitro* model, the particular statin selected and the experimental design, the IC_{50} values of the various statins for the inhibition of HCV replication were shown to be between 1-10 µM for replicon models and 10-30 µM for the HCVcc infectious cell culture system. Most of the DAAs in late clinical development, as well as the two NS3/4A protease inhibitors currently on the market, telaprevir and boceprevir, have IC_{50} values in the low to medium nanomolar range [(Lam et al., 2012), (Lin et al., 2009), (Malcolm et al., 2006), (Paeshuyse et al., 2006), (Perni et al., 2006), (Wang et al., 2012)]. Based on this it can be concluded that statins possess only modest antiviral activity *in vitro* in a monotherapeutic setting.

The possibility of incorporating statins in anti-HCV combination therapy has been investigated by several groups in an *in vitro* setting. Treatment of OR6 HCV replicon cells with a statin (atorvastatin, lovastatin, simvastatin or fluvastatin) in combination with IFN-α resulted in a substantially increased antiviral effect when compared to IFN-α alone (Ikeda et al., 2006). This additive effect was confirmed by our group for combinations of simvastatin or mevastatin with IFN-α (Delang et al., 2009). In addition selected inhibitors of viral HCV replication were combined with these two statins: the NS5B inhibitors 4'-azidocytidine (R1479), benzothiadiazine GSK-4 and benzofuran HCV-796, as well as the NS3/4A protease inhibitor VX-950 (telaprevir). An additive antiviral effect was observed when mevastatin or simvastatin was combined with any of these inhibitors. Furthermore, mevastatin was also able to delay or even prevent the emergence of escape mutants to the non-nucleoside RNA polymerase inhibitor HCV-796. A synergistic antiviral effect of simvastatin in combination with IFN-α in a genotype 1b subgenomic replicon system was also reported (Amemiya et al., 2008). These *in vitro* data suggest that statins may have the potential to (i) increase the efficacy of current or future HCV therapy and (ii) delay the development of resistance against HCV direct-acting antivirals (DAA).

Do statins decrease HCV RNA levels in HCV-infected patients?

a) Monotherapy

In the first clinical trials the potential of statins as antiviral agents for the treatment of HCV was studied in a monotherapeutic setting. Initial pilot clinical trials showed mixed results. In 2007, 10

HCV-infected patients in need of cholesterol-lowering treatment were treated with 20 mg/day of atorvastatin for a period of 12 weeks; HCV RNA levels were measured by PCR at weeks 4 and 12 (O'Leary et al., 2007). When comparing pretreatment HCV RNA levels to HCV RNA levels at weeks 4 or 12, no significant difference was observed. In contrast to this first study, in a small trial conducted in 2008 (n = 22), the authors reported transient, 0.5 $\log_{10}$ reductions in HCV RNA levels in 50% of patients treated with 20-80 mg/day of fluvastatin over a period of 12 weeks, suggesting for the first time that statins can inhibit HCV replication in infected patients (Bader et al., 2008).

Further studies investigating the efficacy of statin monotherapy were conducted and reported largely negative results: a cross-sectional and longitudinal analysis of the response of chronically HCV-infected veterans undergoing statin treatment (n=50) was unable to demonstrate an association between simvastatin therapy and HCV RNA levels (Forde, 2009). A prospective cohort study also failed to document significant changes in HCV RNA levels after 12 weeks of rosuvastatin treatment in 11 chronic HCV-infected patients (Patel et al., 2011). Furthermore, a significant increase in HCV RNA levels was observed in 59% of HIV-HCV co-infected patients after 4 weeks of 80 mg/day fluvastatin treatment (Milazzo et al., 2009). These findings lend credence to the hypothesis that statins may actually induce a pro-viral state *in vivo*, possibly due to the up-regulation of lipoprotein receptors such as low density lipoprotein receptor (LDLR), compensating for the antiviral effects seen *in vitro*. However, this hypothesis has not been confirmed in *in vitro* or *in vivo* studies. In contrast, our unpublished results show no increase of HCV particle uptake in human hepatoma cells following statin treatment. Further studies are required to clarify this matter.

From the above it is clear that statin monotherapy results, at best, in some decrease of viral replication. The modest activity or even lack of an antiviral effect is in stark contrast with the *in vitro* anti-HCV activity of some of the statins. This might be explained by unfavorable pharmacokinetics whereby concentrations required for antiviral activity *in vitro* are not reached in serum following prolonged conventional dosing (20-80 mg/day). For example, the 20 mg daily dose of atorvastatin used in the O'Leary study coincides with a peak serum concentration of 2.5×10^{-3} µM (O'Leary et al.,

2007), which is well below the reported *in vitro* activity range of 1-10 µM. However, as the replication of HCV occurs predominantly in the liver, intrahepatic concentrations of statins may be more relevant than plasma concentrations. Although the thesis that statin concentrations are likely much higher in the human liver than in plasma is widely accepted, there are, to the best of our knowledge, no studies published in which concentrations of statins in the human liver have been determined. In rats, the liver concentration of lovastatin is 15 to 18-fold higher than the concentration in blood and other tissues (Nezasa et al., 2002; Tse et al., 1990; Zhang and Yang, 2007). If one would assume that liver concentrations of statins in the human liver may also be ≈ 15-fold higher than in plasma, a very rough estimation would suggest concentrations of 0.75-15 µM in the human liver (depending on the pharmacokinetic profile of the specific statin). Despite the mostly negative results of statin monotherapy *in vivo*, *in vitro* studies have shown additive, sometimes synergistic, effects of adding statins to the IFN-based standard of care and DAA regimens. It is therefore worth investigating the potential clinical role of statins as a component of anti-HCV combination therapy.

b) Combination therapy

When investigating the antiviral efficacy of statins as a part of combination therapy it is important to distinguish between two different types of studies. On the one hand, a series of retrospective cohort studies have been carried out on patients already taking statins to investigate whether there is a correlation between previous statin use and the efficacy of anti-HCV therapy. A second type of study involves prospective, randomized controlled trials in which a subset of patients are given statins during a set time period to assess whether or not statins improve efficacy of anti-HCV therapy.

i) <u>Retrospective cohort studies – patients already taking statins</u>

In a retrospective study of the IDEAL (Individualized Dosing Efficacy Versus Flat Dosing to Assess Optimal Pegylated Interferon Therapy) trial, concomitant statin use (comprising many different statins, namely atorvastatin, pravastatin, simvastatin, rosuvastatin, lovastatin and fluvastatin) was

found to be a statistically significant positive predictor of sustained virological response (SVR) in HCV genotype 1 infected patients treated with PEG-IFN/RBV (Harrison et al., 2010). SVR is defined as undetectable HCV RNA levels 24 weeks after the cessation of treatment. Statin use was associated with a SVR rate of 53%, as compared to non-statin users who achieved an average SVR rate of 39% ($p = 0.02$). Importantly, this study also showed that there was no significant difference in serious adverse events between statin users and non-statin users. Furthermore, a lower discontinuation rate was reported in the statin user population (38% vs 46% in non-statin users). It should be noted, however, that only a small proportion of the total study population was treated with statins ($n = 66$ vs total $n = 3070$).

Another retrospective analysis of the US Veteran Affairs administrative database investigated multiple predictors of SVR in HCV-infected diabetic patients treated with PEG-IFN/RBV ($n = 8293$) (Rao and Pandya, 2011). Statins were taken by 10.8% of the cohort, of which simvastatin was the most commonly used statin (89%). In a multivariate analysis, concomitant statin use emerged as one of the most positive predictors of SVR in the main cohort studied (OR = 1.39; $p = 0.0007$). Additionally, statin use remained a positive predictor in the diabetic patient cohort (20% of the enrolled patients had diabetes mellitus type 2) included in the study (OR = 1.56; $p = 0.0124$). This study provides a strong, albeit retrospective, indication that statins can be effectively implemented as “adjuvants” to PEG-IFN/RBV combination therapy. Furthermore, statin use appears to be associated with increased SVR in patients with diabetes mellitus type 2, which is in itself a negative predictor of SVR [(Elgouhari et al., 2009)(Konishi et al., 2007)(McHutchison et al., 2009)(Romero-Gómez et al., 2005)]. These data highlight for the first time a potential additional role of statins in the treatment of diabetes patients chronically infected with HCV.

A caveat that requires mentioning when trying to discern a relationship between statin use and antiviral efficacy is the potential confounding effect of the IL28B genotype. It is well established that the rs12979860 CC single nucleotide polymorphism in the IL28B gene is associated with increased SVR rates when patients are treated with IFN-based therapy [(Ge et al., 2009)(Domagalski et al.,

2013)(Ragheb et al., 2013)]. Furthermore, this same polymorphism is also associated with increased serum cholesterol levels in HCV-infected patients (Li et al., 2010). Therefore, patient groups that already take statins as a result of high serum cholesterol, also have a higher chance of possessing the rs12979860 CC IL28B SNP and responding more positively to IFN-based antiviral therapy as a result.

ii) Prospective studies

The first prospective trial investigating the antiviral efficacy of a statin in combination with PEG-IFN/RBV was conducted in 2009 (Sezaki et al., 2009). In a small pilot trial (n = 21) HCV genotype 1b infected patients were administered triple therapy consisting of PEG-IFN/RBV and 20 mg/day fluvastatin administered over a period of 48 weeks. The authors observed a SVR rate of 67%. In comparison, earlier studies of the same patient population where patients received only PEG-IFN/RBV for 48 weeks reported SVR rates of 47-50%. Due to the small number of patients included in this trial, however, no statistically significant differences could be attained. Nonetheless, this study provided an important impetus in the undertaking of further clinical trials to investigate the potential role of statins in anti-HCV combination therapy.

A randomized controlled trial was carried out in 2010 in which 45 patients were treated with either PEG-IFN/RBV or PEG-IFN/RBV in combination with 80 mg/day of fluvastatin and tested for the presence of HCV RNA after 4, 12 and 24 weeks of treatment (Milazzo et al., 2010). In the PEG-IFN/RBV + fluvastatin arm a SVR rate of 38% was reported as compared to a SVR rate in the PEG-IFN/RBV arm of 13%; however this difference was not statistically significant ($p = 0.08$). Interestingly, however, a statistically significant difference in the RVR rate (rapid virological response; defined as negative HCV RNA after 4 weeks of treatment) was reported (33% vs 4%; $p = 0.02$).

A larger randomized controlled trial allocated 101 chronic HCV-infected patients (all infected with HCV genotype 1b) to either a PEG-IFN/RBV treatment arm or a PEG-IFN/RBV + fluvastatin (administered at 20 mg/day) treatment arm for a period of 48-72 weeks (Kondo et al., 2012). The SVR rates achieved in the treatment arms were 41.7% and 63%, respectively ($p = 0.04$). To our knowledge, this is the first randomized controlled study to demonstrate a statistically significant

improvement in SVR rate upon addition of fluvastatin to an IFN-RBV treatment regimen. The authors suggested that statins can be considered as a second line treatment in the event that patients do not tolerate protease inhibitor-based therapies. A retrospective analysis of this study indicated that the mechanism by which fluvastatin increased SVR rates was related to the inhibition of viral relapse, although no biological mechanism for this effect has been proposed (Atsukawa et al., 2013). A more recent meta-analysis of several randomized controlled trials concluded that statins significantly increase SVR rates in combination with PEG-IFN/RBV therapy when administered to patients infected with HCV genotype 1 (Zhu et al., 2013). A summary of the studies discussed above is included in Table 1.

Various studies have shown a correlation between HCV infection and decreased serum lipoprotein levels [(Serfaty et al., 2001)(Petit, 2003)(Siagris et al., 2006)(Hsu et al., 2008)]. The etiology of this hypocholesterolemia is not yet fully understood and is postulated to be either the result of altered lipid metabolism (most likely related to VLDL secretion) in virus infected hepatocytes or hepatic damage resulting from hepatitis (Honda and Matsuzaki, 2011). HCV-associated lipid perturbations also appear to be dependent on HCV genotype. Hypocholesterolemia and steatosis are both strongly associated with HCV genotype 3 infection [(Quadri et al., 2000)(Hui et al., 2002)] and further studies have shown that HCV genotype 3 selectively modulates the cholesterol synthesis pathway [(Jackel-Cram et al., 2010)(Clark et al., 2012)]. Given the fact that statins interact with the same biochemical pathway, their antiviral efficacy may also be genotype-dependent. This genotype variability has not yet been adequately studied, given the fact that most clinical studies to date have been conducted on patients infected with HCV genotype 1 [(Kondo et al., 2012)(Kohjima et al., 2013)]. A recent prospective study investigating treatment efficacy of added FLV to PEG-IFN/RBV therapy in genotype 1 and genotype 3 patients reported no significant increase in SVR rates in the genotype 3 patient population, in contrast to genotype 1 patients with a high viral load, indicating that the antiviral efficacy of statins may indeed be genotype-dependent (Kurincic et al., 2014).

Is the use of statins safe in patients with liver disease?

An important issue facing the implementation of statins as antiviral drugs is the perceived risk of administering lipid lowering drugs to patients with acute or chronic liver disease. Concerns regarding statin safety were raised when cerivastatin was withdrawn from the market in 2001 due to an increased frequency of drug-related rhabdomyolysis and subsequent kidney failure, resulting in 52 deaths worldwide (Furberg and Pitt, 2001). While these side-effects have also been described for the other statins still on the market, they are considerably rarer and estimated to occur at a rate of only 1-3 cases per 100,000 patient years (Law and Rudnicka, 2006). It is therefore generally accepted that the benefits of statin treatment as lipid-lowering therapy far outweigh the risks of severe adverse events and that statins can be considered as an extremely safe class of drugs.

The safety issues associated with the administration of statins to patients with chronic/acute liver disease, however, have been more complicated. Statin treatment can be accompanied by moderate increases in serum ALT and AST values but these effects are generally transient and are not considered to be clinically relevant (Tolman, 2000). In addition, while isolated case reports of statin-related hepatoxicity have been described (Clarke and Mills, 2006; Kinnman and Hultcrantz, 2001), these events are extremely rare and no clear association between statin use and the onset of liver disease has yet been reported (de Denus et al., 2004; Law and Rudnicka, 2006). Several studies have been conducted to investigate the safety profile of statins in patients chronically infected with HCV. No significant elevations in ALT or AST could be measured in 17 HCV-infected patients taking simvastatin (Gibson and Rindone, 2005). A larger study was carried out in which changes in liver enzymes were investigated over a 12-month period in three distinct patient cohorts: i) HCV-infected patients not undergoing statin therapy, ii) HCV-infected patients undergoing statin therapy and iii) non HCV-infected patients undergoing statin therapy (Khorashadi et al., 2006). Importantly, while statin therapy in HCV-infected patients was associated with a greater incidence of mild-moderate increases in liver enzymes as compared to HCV-infected patients not undergoing statin therapy, these increases were not found to be significantly different to the liver enzyme elevations measured

in non HCV-infected patients undergoing statin therapy. This seems to suggest that HCV-infected patients taking statins are at no greater risk of hepatotoxicity as compared to uninfected hyperlipidemic patients i.e. the main patient population taking a statin.

While these studies support the postulation that statin therapy is safe in HCV-infected patients it is important to note that many of these studies were undertaken to assess safety of statin therapy for the purpose of lowering LDL levels. The optimal dosage regime required for the successful treatment of HCV may be different and it is important to assess the safety of statin treatment in this setting as well. Nevertheless, these studies provide evidence that statin treatment should be considered safe in HCV-infected patients.

Given the fact that HCV-infection is associated with hypocholesterolemia it is possible that further lowering cholesterol levels with statin add-on therapy may exacerbate side-effects associated with this disorder. This would be especially relevant in patients infected with HCV genotype 3, given the stronger association of this genotype with perturbations in lipoprotein levels. A notable side-effect in this regard is depression; several studies have reported an inverse correlation between serum cholesterol levels and the incidence of depression and suicidal ideation in both population and primary care settings (reviewed in Sansone and Sansone, 2008). More recent studies have also demonstrated that chronic HCV infection itself [(Carta et al., 2012)(Lee et al., 2013)] as well as interferon-based anti-HCV therapy (Udina et al., 2012) are both associated with an increased incidence of depression in HCV-infected patients. Given these data it is tempting to conclude that cholesterol-lowering statin add-on therapy would further increase the risk of depression in chronic HCV patients already taking interferon-based therapy. However, the effects of statins themselves on the incidence of depression remain unclear, as shown by a recent meta-analysis of 7 studies surrounding this topic in which an anti-depressive effect of statin therapy was reported (Parsaik et al., 2013). Further studies are certainly warranted to ascertain what the effect will be of adding statins to established anti-HCV therapy regarding the incidence of depression in chronic HCV patients.

When proposing a role for statins in anti-HCV combination therapy, it is important to take potential drug-drug interactions into account. Statins are documented cytochrome P450 (CYP) isoenzyme substrates, the extent of metabolism varying for each statin (Causevic-Ramosevac and Semiz, 2013). Lovastatin, simvastatin and atorvastatin are primarily metabolized by CYP3A4 and are most prone to drug-drug interactions with CYP3A4 inhibitors, which can increase the risk of statin-associated adverse events. Both anti-HCV protease inhibitors currently on the market, boceprevir and telaprevir, are potent inhibitors of CYP3A4 and are thus especially prone to drug-drug interactions (Kiser et al., 2013). Telaprevir has been shown to substantially increase the plasma concentrations of atorvastatin in patients (Lee et al., 2011). Statin-associated adverse events have been described for patients taking HIV protease inhibitors, which are also potent CYP3A4 inhibitors (Schmidt et al., 2007). For this reason, lovastatin, simvastatin and to some extent atorvastatin are generally contraindicated for concomitant use with telaprevir or boceprevir (Burger et al., 2013; Chauvin et al., 2013; Teixeira et al., 2013). It is important to note, however, that the most active statin *in vitro*, fluvastatin, is primarily metabolized via CYP2D9 and, along with pravastatin, is generally considered safe for combination with CYP3A4 inhibitors (Causevic-Ramosevac and Semiz, 2013; Chauvin et al., 2013). The CYP inhibition profile will have to be carefully ascertained for the newer anti-HCV drugs in late clinical development in order to assess the potential for statin combination therapy.

Perspectives

With new and highly efficacious DAAs approaching market approval, future prospects for the treatment of patients with chronic HCV infection appear favorable. Nevertheless, an issue which is easily forgotten in this regard is the rising cost of DAA-based combination therapy. Telaprevir- and boceprevir-based therapies are estimated to cost up to $50,000 per treatment course, with the protease inhibitor comprising almost half of this amount (Gellad et al., 2012), (http://www.hepatitiscnewdrugresearch.com/cost-of-treating-with-telaprevir.html, last consulted: 19-02-2014) and drugs such as Sofosbuvir (Gilead) and Simeprevir (Johnson & Johnson) are at least as

expensive. While developed nations may be able to afford these costly regimens, the situation is drastically different in the developing world. A significant portion of HCV-infected patients worldwide require treatment in a limited resource setting and will most likely not benefit from expensive DAA-based therapies in the immediate future. Adding statins to the established IFN-based combination therapy in these countries, where applicable, could serve to increase SVR rates until the time that the newer, more efficacious DAAs become available and affordable. The patents for fluvastatin, atorvastatin, simvastatin and lovastatin have expired and all these drugs are currently available as generics, substantially lowering their cost. A recent report published by the WHO also suggested a role for statins in the treatment of cardiovascular disease in developing nations, indicating administration of these drugs in resource limited countries is indeed feasible (Prevention and Control of Noncommunicable Diseases: Guidelines for primary health care in low-resource settings, WHO report, 2012). Additionally, while this WHO report investigated the use of statins in a cardiovascular disease setting, treatment duration for anti-HCV therapy would be substantially shorter, typically limited to 12-24 weeks, which would decrease the costs of administering statins for this indication in developing nations.

Statins possess only modest anti-HCV activity *in vitro* and appear ill-suited as an anti-HCV drug administered as monotherapy to HCV-infected patients. However, in combination with IFN-based therapy, significant increases in SVR rates can be achieved. This does not seem sufficient, however, to justify including statins in future DAA-based combination regimens, particularly when taking into account that a series of promising combinations of direct-acting HCV drugs will achieve market approval in the coming years. However, the excellent safety profile of statins, the availability of generics as well as the high cost of current and future DAAs are factors which highlight the potential of statins in resource limited settings. Furthermore, as statins could be able to improve SVR rates at an affordable cost, these drugs may fulfill an important role in “bridging the gap” until more potent and cheaper DAAs become available.

Acknowledgements. Ben Verpaalen is a fellow from the IWT (Instituut voor Wetenschap en Technologie, - Vlaanderen) and Leen Delang is a post-doctoral fellow from the FWO (Fonds voor Wetenschappelijk Onderzoek – Vlaanderen). The original work of the authors is supported by a grant from the IWT (SBO ####) and from Belspo (Interuniversitaire attractiepolen) (SBO ####) *(SBO numbers still to be added).*

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597 Table 1: Overview of clinical studies investigating use of statins as antiviral agents against HCV

MONOTHERAPY							
Author (year)	**Study design**	**No. of patients**	**HCV genotype (n)**	**Statin use, dose (n)**	**Control**	**Duration (weeks)**	**Outcome**
O'Leary et al (2007)	Prospective study	10	1 (8) 2 (1) 4 (1)	Atorvastatin, 20 mg/day (10)	NA	12	No correlation between atorvastatin use and post-treatment HCV RNA levels
Bader et al (2008)	Prospective study	31	1 (15) 2 (9) 3 (7)	Fluvastatin, 20-80 mg/day (10)	NA	12	Transient 0.5$\log_{10}$ reductions in 50% of patients after fluvastatin treatment
Forde et al (2009)	Retrospective cohort study	50	1 (34) 2 (4) 3 (1) Not typed (1)	Simvastatin, NA (42) Lovastatin, NA (5) Pravastatin, NA (2) Fluvastatin, NA (1)	NA		No correlation between statin use and post-treatment HCV RNA levels
Patel et al (2011)	Prospective cohort study	11	1 (11)	Rosuvastatin, 20-40 mg/day (11)	NA	12	No correlation between rosuvastatin use and post-treatment HCV RNA levels
Milazzo et al (2009)	Randomized controlled study	43	1 (43) *HIV coinfection	Fluvastatin 80 mg/day (22)	Control arm: no treatment (21)	4	Positive correlation between statin use and post-treatment HCV RNA levels (p = 0.032)
COMBINATION THERAPY							
I. Retrospective cohort studies: patients already taking statins							
Author (year)	**Study design**	**No. of patients**	**HCV genotype (n)**	**Statin use, dose (n)**	**Combination regimen (n)**	**Duration (weeks)**	**Outcome**
Harrison et al (2010)	Retrospective data analysis	3070	1 (3070)	Atorvastatin, NA (29) Pravastatin, NA (14) Simvastatin, NA (10) Rosuvastatin, NA (6) Lovastatin, NA (5) Fluvastatin, NA (1) Ezetimibe/simvastatin, NA (1)	+ PEG-IFN alfa2b, 1.5 µg/kg/RBV (22) + PEG-IFN alfa2b, 1 µg/kg/RBV (22) + PEG-IFN alfa2a /RBV (22)	48	Positive association between statin use and SVR rate (p = 0.02)
Rao and Pandya (2011)	Retrospective data analysis	8293	Unknown	Simvastatin, NA (798) Others , NA (99)	+ PEG-IFN/RBV (8293)	-	Positive association between statin use and SVR rate (p = 0.0007)
II. Prospective studies							
Sezaki et al (2009)	Prospective study	21	1b (21)	Fluvastatin, 20 mg/day (21)	+ PEG-IFN/RBV (21)	48	No statistically significant association between statin use and SVR rate
Milazzo et al (2010)	Randomized controlled study	44	1 (44)	Fluvastatin, 80 mg/day (22)	+ PEG-IFN/RBV (44)	24	Positive association between statin use and RVR rate (33% vs. 4%; p = 0.02)
Kondo et al (2012)	Randomized controlled study	94	1b (94)	Fluvastatin, 20 mg/day (46)	+ PEG-IFNalfa2b 1.5 µg/kg/week / RBV 600-1000 mg/day (94)	48-72	Positive association between statin use and SVR rate (63% vs. 41.7%; p = 0.04)

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